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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/816,099	03/31/2004	Katalin Varadi	P-279.00	9454

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EXAMINER	
KOSSON, ROSANNE	
ART UNIT	PAPER NUMBER
1652	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/816,099	VARADI ET AL.	
	Examiner	Art Unit	
	Rosanne Kosson	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *21 October 2009*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-8,10-13,22 and 23* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-8,10-13,22 and 23* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicants' amendment filed on October 21, 2009 has been received and entered. Claims 1, 22 and 23 have been amended. Claims 9, 14-21 and 24 have been canceled. No claims have been added. Accordingly, claims 1-8, 10-13, 22 and 23 are examined on the merits herewith.

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claim Rejections - 35 USC § 112

In view of Applicants' amendments to the claims, the rejection in the previous Office action is withdrawn. As discussed in the previous Office action, however, the "wherein" clauses in claim 22 step (a) are possible uses or intended uses of the lyophilized substrate-$CaCl_2$-DMSO mixture, but they are not assay steps. The same is true of the second "wherein" clause in claim 1, as water is not part of the kit, because component (ii) is a lyophilized reagent.

Claim Rejections - 35 USC § 103

Claims 1-8, 10-13, 22 and 23 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Wöber et al. (US 6,124,110) in view of Hawkins et al. (US 5,625,036), Váradi et al. ("Monitoring the bioavailability of FEIBA with a thrombin generation assay," J Thrombosis and Heamostasis 1:2374-2380, 2003), Chan (US 5,952,198), Hogan et al. (US 6,074,826), Weinstein et al. (US 6,576,422) and Dubrow et al. (US 6,756,019). This rejection has been discussed in the previous Office actions. Lawson et al., Dou et al. and CRC (CRC Handbook of Chemistry and Physics 51st Ed.) have been dropped from the rejection, because the claims

have been amended to recite only one fluorescently labeled thrombin substrate (Z-G-G-R-AMC), not any fluorescently labeled thrombin substrate.

To reiterate, Wöber et al. disclose reagents and an assay for measuring thrombin generation in plasma samples. They disclose natural tissue factor (TF) as a dry powder and solutions of the phospholipids phosphatidylserine (PS) and phosphatidylcholine (PC). Wöber et al. also disclose that these three reagents are combined to prepare a solution of vesicles or liposomes containing TF, i.e., a TF/PL complex. The ratio of PC to PS in this complex is 6:4. This solution may be frozen in assay portions (see col. 2, lines 15-26; col. 3, lines 9-16, and col. 4, lines 13-46). Wöber et al. also disclose a thrombin standard that is used in their assay (see col. 5, lines 20-40) (see claims 1, 6, 7, 10, 22 and 23). Wöber et al. do not disclose that this complex is lyophilized or the concentrations of TF and PL in the complex.

Hawkins et al., however, disclose that the TF/PL solution may be lyophilized. The ratio of PC to PS in Hawkins et al. is 7:3 (see Example 2 in col. 8 and Example 4 in cols. 9 and 10). The ratio of TF to PL is 1:2000 to 1:20,000, and the PL concentration is 1-300 μM (see col. 4, lines 34-50). At a PL concentration of 1 μM, the TF concentration is 50-500 pM. Synthetic (recombinant) or natural TF may be used, and synthetic or natural phospholipids may be used. Combinations of lipids other than PC and PC may be used (see col. 4, lines 34-62, and col. 5, lines 3-10) (see claims 1-5).

One of ordinary skill in the art at the time of the invention would have been motivated to lyophilize the TF/PL preparation of Wöber et al., because Hawkins et al. teach that this preparation is made as a reagent for performing assays to measure prothrombin time (see Title). This reagent is meant to be used for large-scale clinical assays and is designed to have minimal variability from lot to lot (see col. 1, line 36, to col. 2, line 7. One of ordinary skill in the art would have recognized that a manufacturer of such a reagent would have lyophilized it to

reduce the weight and volume for shipping purposes and to impart stability to the reagent. Dry materials are less susceptible to degradation than their liquid form, e.g., as with powdered vs. liquid milk.

One of ordinary skill in the art would have been motivated to prepare a TF/PL complex with the TF and PL concentrations and with the phospholipid ratios disclosed in Hawkins et al., because Hawkins et al. teach that these are suitable amounts for preparing a reagent for an assay for measuring prothrombin time. Prothrombin is a thrombin precursor in the clotting pathway (cleaved by the protease prothrombinase to form thrombin). Thus a reagent for measuring prothrombin time may also be used to measure thrombin time.

Regarding the lyophilized thrombin substrate and $CaCl_2$ preparation, Wöber et al. disclose a dry chromogenic thrombin substrate, S 2238 (Chromogenix, now Diapharma) that is soluble in water and that the thrombin reaction is initiated by the addition of $CaCl_2$ to the assay samples (see col. 5, lines 10-25). One of ordinary skill in the art would have been motivated to prepare a lyophilized reagent containing thrombin substrate and $CaCl_2$, because Hawkins et al. teach the advantages of lyophilized reagents in clinical assays. As noted above, the artisan of ordinary skill would have recognized that aqueous solutions can be lyophilized to reduce bulk and improve stability. One of ordinary skill in the art would also have recognized that the thrombin substrate and $CaCl_2$ are both soluble in water or buffer, as disclosed by Wöber et al., and they would have been combined because an enzymatic reaction may also be initiated by the addition of substrate, as well as by the addition of a catalytic substance or cofactor. In an assay of a number of samples, the enzymatic reaction is initiated by the addition of a reagent, ideally simultaneously to all samples, but this reagent may contain the substrate and the cofactor. Combining the substrate and the cofactor reduces the number of pipetting steps, thereby reducing the chance of assay errors due to pipetting errors, and reduces the number of

assay steps, allowing the assay to be performed faster. Because the thrombin substrate and $CaCl_2$ are both soluble in water or buffer, one of ordinary skill in the art would have recognized that a solution containing both of these substances would have been prepared and lyophilized. Moreover, Hogan et al. teach that, in a diagnostic kit, or when performing an assay with a diagnostic kit, the reagents may be premixed before lyophilization so that, when reconstituted, a complete mixture is formed with the reagents in the proper ratio and ready for use (see col. 37, lines 14-29) (see claim 1).

Regarding a thrombin substrate containing a fluorescent label, as noted above, Wöber et al. disclose a substrate containing a colored label. Determinations of thrombin generation are made by measuring the extinction over five minutes at one minute intervals at 405 nm (see col. 5, lines 33-40). Váradi et al., however, disclose a thrombin substrate for a thrombin generation assay that contains a fluorescent label, Z-Gly-Gly-Arg-AMC. An assay reagent comprising 1 mM thrombin substrate and 15 mM calcium chloride was prepared. In each assay sample, 10 µl of a preparation containing a TF/PL complex (the complex containing 17.9 pM TF and 3.2 µM PL, the PL being PC:PS, 80:20) was added to 50 µl of the thrombin substrate solution, and 40 µl of plasma was added to start the reaction. In the assay samples, increases in fluorescence were measured every minute over 2 hours at 460 nm (see p. 2375, Thrombin generation assay). One of ordinary skill in the art would have been motivated to use the thrombin substrate of Váradi et al. as the thrombin substrate in the set of reagents disclosed by Wöber et al., i.e., a fluorescent label instead of a colored label, because Váradi et al. teach that their substrate is available as a dry powder that is soluble in the buffers used in a thrombin generation assay. Thus, lyophilized forms of these powders may also be prepared. One of ordinary skill in the art would have recognized that these substrates are interchangeable with the substrate of Wöber et al., as it would have been well within his capability, when performing a thrombin generation

assay, to measure the amount of fluorescence produced over time by a thrombin reaction product instead of the amount of color generated over time by a thrombin reaction product. Both fluorometric and spectrophotometric measurements are standard assay techniques (see claims 1-3, 6, 7, 22 and 23).

With respect to claim 8, which recites phospholipids comprising PC, PS and PE (polyethanolamine) in a ratio of about 60:20:20 to about 78:17:5, the composition of phospholipid mixtures and the ratios of the different lipid components are result-effective parameters which were routinely optimized by one of ordinary skill in the art. Thus, the claimed variations in Applicants' composition with respect to these parameters clearly would have been obvious at the time of Applicants' invention, the optimization of these parameters being well within the capabilities of the artisan of ordinary skill at the time of Applicants' invention. Additionally, liposomes comprising such a lipid mixture were known at the time of Applicants' invention. Chan (US 5,952,198) discloses liposomes of PC, PS, and PE in a ratio of 4:1:1 that are added to the medium of 293S cells (human embryonic kidney cells) to increase the production of recombinant Factor VIII, a clotting factor. Liposomes of PC, PS and PE in a ratio of 8:1:1 and 16:2:1 are also disclosed (see Table 1, cols. 3 and 4). It is thought that these liposomes stabilize the recombinant Factor VIII in an in vitro medium (see col. 1, lines 49-52). The liposomes with a ratio of 4:1:1 and 16:2:1 are close to those in Applicants' claimed range. One of ordinary skill in the art would have been motivated to use liposomes comprising PC, PS and PE in a ratio of about 60:20:20 to about 78:17:5 instead of liposomes comprising PC and PS in a ratio of 6:4 in a complex with TF because Chan teaches that the PC:PS:PE liposomes have a stabilizing effect on clotting factors, Factor VIII and von Willebrand factor. As noted above, Hawkins discloses that lipid mixtures other than PC:PS, 6:4, may be used in the TF/PL complex.

Regarding a solid support, such as microtiter plate, with lyophilized assay reagents coated onto the wells or assay vessels of the support, and a method of performing an assay using this solid support, Weinstein et al. disclose an assay method using a solid support such as a microtiter plate in which lyophilized detection reagents are immobilized on the solid support (see col. 16, lines 10-25, and col. 17, lines 5-6). The advantages of performing an assay with this solid support are that the assay is fast and simple and designed for screening a large number of samples (see col. 17, lines 22-30). Dubrow et al. also disclose performing an assay with minute amounts of lyophilized reagents immobilized on a solid support (see col. 12, line 59, to col. 13, line 11). Current trends in biochemical analyses have been toward miniaturization, particularly in microfluidics systems (which manipulate microtiter plates), and which have the advantages of small amounts of reagents needed, faster throughput, automation and improved data (see col. 1, lines 12-19). One of ordinary skill in the art would have been motivated to prepare a kit for measuring thrombin generation comprising assay reagents lyophilized and immobilized on a solid support, because Hawkins et al. teach the advantages of lyophilized reagents, and Weinstein et al. and Dubrow et al. teach the advantages of formulating these reagents as a lyophilized coating on a solid support. Weinstein et al., more specifically, teach the advantages of formulating assay reagents as a lyophilized coating on the wells of a microtiter plate. The cited references also teach the benefits of performing assays with these solid supports (speed, simplicity, high throughput, improved data) (see claims 11-13).

Applicants assert that the claimed invention is not obvious, because Váradi et al. are not prior art, based on Applicants' Declaration under 37 CFR §1.132, filed with Applicants' Response. In reply, Váradi et al. are prior art. The reference was published in November 2003, and Applicants have a priority date of March 31, 2004. The reference has nine authors,

although three of them, Váradi, Schwarz and Turecek, are inventors in the instant application, which has five inventors. Thus, Váradi et al. are a different entity than the inventors of the instant application. Applicants have disclaimed the authors who are not inventors as individuals who did not contribute intellectually to the publication, stating that that these six people merely provided blood samples. But, the group of the remaining three people is a different inventive entity than the five instant inventors.

Generally, a reference may be disqualified as prior art in one of three ways. The inventorship may be changed to match the authors of the reference. Alternatively, the reference may be antedated with supporting evidence in a Declaration under 37 CFR §1.131. Or, Applicants may show, with concrete supporting evidence (such as notebook pages or notes and slides from meetings), that inventors who are not authors could have been authors, i.e., that they contributed intellectually and substantively to the subject matter published in the reference. They provided certain key ideas and explained how the studies to test or prove these ideas should be carried out. But, Applicants have explained in their Declaration that Inventors Keil and Peyrer-Heimstätt did not contribute to the subject matter of the reference. Thus, the third option is not available. Because the reference is still prior art, a holding of obviousness is still required.

No claim is allowed.

Applicants' amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicants are reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO

MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Rosanne Kosson whose telephone number is (571)272-2923. The examiner can normally be reached on Mon., Thurs., Fri., 8:30-6:00, Tues., 8:30-2:00, Wed. off.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Andrew Wang, can be reached on 571-272-0811811. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Rosanne Kosson
Examiner, Art Unit 1652
rk/2009-12-07

/Karen Cochrane Carlson/
Primary Examiner, Art Unit 1656